

<p>Biomechanical Orthopedics Laboratory EPFL-HOSR &amp; Orthopedic Cell Therapy Unit CHUV</p> <p><b>SOP 08-05</b></p> <p>Page 1 of 1</p>	<p><b>Cell culture</b> (Changing media)</p> <p>Version 4</p>	<p>Creation date : 12.11.03 Created by : DP, AK Modification date : 18.12.07 Modified by : LALA</p>
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**Materials :**

- DMEM (with L-Glutamine, 4,5 g/l D-Glucose and Sodium pyruvate) 500 ml (Invitrogen, #41966-29) at 4°C
- Fetal Bovine Serum (FBS) 500 ml -> aliquot 50 ml (Sigma, #F7524) at -20°C
- L-Glutamine (200 mM ;100x) 100ml -> aliquot 5 ml (Invitrogen #25030-024) at -20°C

**Culture medium preparation : (for 500ml)**

- Add 50 ml FBS(10%), 5 ml L-Glutamine (1%) to the 500ml DMEM bottle
- Filter, if necessary, the medium with Stericup system (Milian, SCGPU05RE)
- Heat the culture medium, PBS and Trypsin at RT or in 37°C water bath

**Procedure :**

1. Clean and prepare inside the hood and all instruments
2. Check the cells with the microscope and the sterility
3. Throw away the culture medium
4. Put fresh culture medium 4 ml for small flasks (25 cm<sup>2</sup>) , 10 ml for flasks (80 cm<sup>2</sup>), 7-10 ml for culture plate (Ø 10 cm and 3 ml for Ø 6 cm )
5. Place in the incubator at 37°C and 5% CO<sub>2</sub>.
6. Culture medium changes 2-3 times per week